Progression and Regression of Atherosclerotic Lesions
Monitoring With Serial Noninvasive Magnetic Resonance Imaging

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Background—Modification or stabilization of atherosclerotic plaques has been proposed as the mechanism responsible for the beneficial clinical effect of lipid-lowering therapies. An imaging modality able to quantify atherosclerotic plaque composition could potentially allow not only the identification of these vulnerable atherosclerotic lesions, but also monitoring of the effects of therapeutic interventions on plaque composition. Our aim was to monitor changes in burden and composition of atherosclerotic plaques in a rabbit model of complex atherosclerosis using serial noninvasive magnetic resonance imaging (MRI).

Methods and Results—Aortic atherosclerotic lesions were induced in rabbits and the animals randomized to continue an atherogenic diet (atherosclerosis progression) or resume normal chow (atherosclerosis regression) for 6 months. MRI of the aorta was performed at 3 time points in each rabbit, as follows: baseline, after atherosclerosis induction (9 months old), and after atherosclerosis regression or progression (15 months old). Histopathologic correlation with MRI was performed. There was a significant (P<0.0001) reduction in atherosclerotic burden in the regression group, and an increase in the progression group. There was a significant (P<0.001) reduction in the lipidic component of plaques in the regression group, and an increase in the progression group. A small, nonsignificant increase in the fibrotic plaque components was noted in the regression group, but a significant decrease in the fibrotic composition of lesions in the progression group. A significant correlation (P<0.001) was found between MRI and histopathology for atherosclerotic burden and plaque composition.

Conclusions—These findings indicate that serial noninvasive MRI can monitor changes in atherosclerotic plaque composition under conditions of atherosclerotic progression and regression. (Circulation. 2002;105:993-998.)

Key Words: magnetic resonance imaging ■ atherosclerosis ■ remodeling

The pathogenesis of the acute coronary syndromes is frequently related to atherosclerotic plaque disruption and subsequent thrombosis.1,2 Atherosclerotic plaque composition, rather than the degree of arterial stenosis, appears to be a critical determinant of both risk of rupture and subsequent thrombogenicity.3 In particular, a large lipid core and a thin fibrous cap render a lesion susceptible or vulnerable to disruption and thrombosis.3,4 Modification or stabilization of the vulnerable plaques in the coronary arteries, by strengthening the fibrous cap and decreasing the lipid core, has been proposed as an important mechanism responsible for the observed beneficial clinical effect of these lipid-lowering therapies.5,6 Therefore, an imaging modality able to noninvasively quantify atherosclerotic plaque composition could potentially allow not only the identification of these vulnerable atherosclerotic lesions, but also monitor effects of therapeutic interventions for stabilization on the plaque composition.7

Magnetic resonance (MR) imaging (MRI) is able to quantify and characterize noninvasively lesions in humans and animal models of atherosclerosis.8–14 We have recently shown that in vivo noninvasive MRI accurately quantifies fibrotic and lipidic components of atherosclerosis in rabbits.15 The ability of MR to visualize changes in atherosclerosis over time has been reported13; however, the specific ability of MR to quantify the changes in both atherosclerotic burden and plaque composition over time has not been shown. This advance could allow the serial monitoring of a given plaque over time, thus permitting studies of therapies for atherosclerotic plaque stabilization.
We report the ability of serial noninvasive MRI to monitor changes in lipidic and fibrotic components of the same atherosclerotic plaques as well as plaque size in a rabbit model, in response to dietary modification.

Methods

Experimental Design: Atherosclerosis Progression and Regression

Atherosclerotic aortic lesions with fibrotic and lipidic components were induced in New Zealand White rabbits (3.0 to 3.5 kg, \(n=15\); Covance, Princeton, NJ) as previously described.\(^{15,16}\) We euthanized a subgroup of animals (\(n=5\)) after atherosclerosis induction for validation of MRI with histopathology at this time point. This same subgroup has been analyzed previously and has permitted the demonstration of the feasibility of in vivo, noninvasive, high-resolution MRI to quantify lipidic and fibrotic components in the aorta.\(^{15}\) After 6 months of the atherogenic diet (weight 3.2 to 3.8 kg) (atherosclerosis induction), the remaining animals were randomized either to continue the atherogenic diet (atherosclerosis progression, \(n=5\), final weight 3.4 to 4.1 kg) or to resume normal chow (atherosclerosis regression, \(n=5\), final weight 3.3 to 3.9 kg) for a further 6 months. Thereafter, these animals were also euthanized for comparison between the MR images and histopathology. All experiments were approved by the Mount Sinai School of Medicine animal management program.

Magnetic Resonance Imaging

Serial MRI was performed at the following 3 time points: baseline, atherosclerosis induction, and atherosclerosis progression or regression. Gradient-echo coronal images were used to localize the abdominal aorta. Thereafter, sequential axial images (3-mm thickness) of the abdominal aorta from the renal arteries to the iliac bifurcation were obtained using a fast spin-echo sequence as described previously by us\(^{15,16}\) and others\(^{11}\) (total imaging time 35 minutes) with an in-plane resolution of 350×350 μm (proton density weighted [PDW], repetition time/echo time [TR/TE] = 2300/17 ms; \(T_2\) weighted [T2 W], TR/TE = 2300/60 ms, field of view 9×9 cm, matrix 256×256, echo train length 8, and signal averages 4). Inferior and superior radiofrequency saturation pulses were used to null the signal from flowing blood in the inferior vena cava and aorta. Fat suppression was used to null the signal from the perivascular fat.

Image and Data Analysis

The MR images were transferred to a Macintosh computer and matched with the corresponding histopathologic sections for the aortic specimens. Cross-sectional areas of the lumen and outer boundary of each aortic section were determined for both MR images and histopathology by manual tracing with ImagePro Plus (Media Cybernetics). The outer boundary was defined as the vessel wall–epicardial fat interface. From these measurements, vessel wall area (VWA) and lumen area were calculated. Mean wall thickness (MWT) was derived by automated analysis of the lumen and outer vessel area using ImagePro Plus (Media Cybernetics).
Areas containing lipidic and fibrotic material were also measured, using T2W images. The ability of MRI to quantify these regions with computer-assisted morphometry has been recently reported. The study design allowed for the serial comparison between MR images from the same animals at the same aortic site (as confirmed by distance from the renal arteries and the iliac bifurcation) over the 3 time points. Thus, serial data about changes in the size and composition of the vessel wall with MRI could be analyzed. Histopathologic measurements of MWT and VWA were analyzed with sections stained by combined Masson elastin stain (CME); lipidic and fibrotic areas were analyzed with Oil Red O staining. An independent investigator blinded to the results of MR findings performed the histopathologic analyses.

### Statistical Analysis
The correlations between measurements of MWT, VWA, and lipidic and fibrotic area by MR and histopathology were analyzed by simple linear regression with 95% confidence intervals (Statview, SAS Institute, Inc). Comparisons of MR images of the same aortic sections from the same animals between different time points were made using repeated-measures ANOVA techniques, with Bonferroni-Dunn post hoc testing performed where appropriate. Serial analysis of lipidic and fibrotic areas with MRI was performed using paired Student t testing (data for 2 time points exist). Comparisons between groups for MR images and histopathologic stains were performed using unpaired Student t testing. All values are expressed as mean±SEM. A P value <0.05 was used to indicate statistical significance, and all statistical tests were 2-tailed.

### Results
#### Serial MRI of Vessel Wall Parameters
There was a significant increase in the atherosclerotic burden after the 9-month induction period as compared with baseline MR (P<0.0001, n=70 sections) (Figures 1 to 4). Three animals died during the study period (2 in the progression group and 1 in the regression group) and were subsequently not analyzed. The VWA and MWT increased from 4.62±0.09 to 7.59±0.26 mm² and 0.38±0.01 to 0.65±0.02 mm, respectively (P<0.0001), as determined by serial MRI.

Comparison of vessel wall parameters determined by serial MRI in animals that were returned to a normal chow diet after atherosclerosis induction confirms that there is a significant (P<0.0001 for VWA; P=0.0006 for MWT, n=40 sections) reduction in atherosclerotic burden in this group (Figures 1 and 2). Serial MRI at the 3 time points in these animals confirms the progression and regression of atherosclerosis in the same animals (Table). Similarly, in animals randomized to continued atherosclerosis progression, there was a further significant increase (P<0.0001 for both VWA and MWT, n=30 sections) in atherosclerotic burden as determined by measurements of vessel wall parameters (Figures 3 and 4 and Table). The histopathologic sections allow us to appreciate both the differential in plaque burden as well as composition between the regression and progression groups (Figure 5).

There was a small but statistically significant (P<0.0001) difference in aortic lumen area at baseline in animals subsequently randomized to the progression and regression arms (Table). Interestingly, after atherosclerosis induction (9 months old), there was a subsequent negative remodeling noted in the atherosclerosis regression group (outer VWA percentage decrease of 17.6%, P<0.0001) and subsequent positive remodeling in the atherosclerosis progression group.

### Table: Serial MRI of Vessel Wall Parameters

<table>
<thead>
<tr>
<th></th>
<th>AT Prog</th>
<th>AT Reg</th>
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<tr>
<td>Lumen Area, mm²</td>
<td>9.56</td>
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<td>15 Months</td>
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<td>0.54</td>
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<td>6.21</td>
<td>1.92</td>
<td>0.13</td>
<td>4.15</td>
<td>5.58</td>
</tr>
</tbody>
</table>

AT Prog indicates atherosclerosis progression group; and AT Reg, atherosclerosis regression group.

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*Figure 3.* Serial T2W MR images of atherosclerosis in rabbit abdominal aorta taken at baseline (A and D), 9 months (B and E), and 15 months (C and F) from the same rabbit from the progression group at 2 different levels (at the first spinal artery in A, B, and C and at the left renal artery in D, E, and F).
(outer VWA percentage increase of 6.5%, \( P = 0.047 \)). This is consistent with previous work in the Watanabe Heritable Hyperlipidemic rabbit. \(^{16}\)

Serial MRI of Plaque Composition

MRI of animals after atherosclerosis induction confirmed the presence of significant aortic atherosclerosis with both fibrotic and lipidic components identifiable (mean area of the fibrotic component, \( 5.38 \pm 0.34 \text{ mm}^2 \); mean area of the lipidic component, \( 0.93 \pm 0.19 \text{ mm}^2 \)). Serial imaging after atherosclerosis progression or regression thus allowed the study of plaque component changes over time.

In the atherosclerotic regression group, at 15 months, there was no significant change (\( P = 0.49 \)) in the fibrotic component of the atherosclerotic lesions (percentage of fibrotic component from 82% to 97%), although there was a trend toward an increase. However, there was a significant (\( P < 0.001 \)) decrease in the lipidic component of these lesions (percentage of lipidic component from 18% to 2%) (Table).

In contrast, at 15 months, in the atherosclerosis progression group, there was a small but significant (\( P < 0.05 \)) decrease in the fibrotic component of the lesions (percentage of fibrotic component from 88% to 68%). Furthermore, there was a significant increase (\( P < 0.01 \)) in the lipidic component (percentage of lipidic component from 10% to 32%) (Table).

Correlation Between MRI and Histopathology

There was good agreement between MRI and histopathology (\( n = 120 \) sections) for the measurement of both VWA (\( r = 0.81, P < 0.001 \)) and MWT (\( r = 0.85, P < 0.001 \)) as assessed by simple linear regression analysis.

There was excellent agreement between MRI and histopathology for atherosclerotic composition, allowing tracing of both lipidic and fibrotic regions by both MRI and histopathology (Figure 6). Furthermore, significant agreement existed between MR and histopathologic measurements of lipidic (\( r = 0.94, P < 0.01 \)) and fibrotic (\( r = 0.79, P < 0.01 \)) areas for the subset of aortic sections stained with Oil Red 0 (\( n = 14 \) sections, 2 sections per rabbit) (Figure 7), confirming previous studies. \(^{15}\) The sections for Oil Red O staining included the section immediately proximal to the left renal artery, and the section immediately distal to the last (ie, tenth) section of aorta that was used for CME staining.

Figure 4. Magnification of Figure 3 demonstrating co-registration of serial images from the same rabbit of the progression group. Arrow indicates spinal artery in A, B, and C and left renal artery in D, E, and F. There is an increase in aortic atherosclerotic burden after the 9-month induction period and a further increase at 15 months. Within the large, complex atherosclerotic lesions in the 15-month group (C and F), one can appreciate the higher signal (brighter) of the fibrous cap and the lower signal (darker) of the lipidic core with the T2W MR image.

Figure 5. Pathological sections from the regression group (A, B, and C) and the progression group (D, E, and F) at the level of the left renal artery (A and D) and mid-abdominal aorta (B and E), taken at the same distance from the left renal artery in each case for comparability. Staining is with CME. C and F are magnifications of the atherosclerotic lesions from B and E, respectively, showing large numbers of smooth muscle cells with collagen in the regression group and abundant macrophage foam cells and associated cholesterol clefts in the progression group. The images allow us to appreciate both the differential in plaque burden and composition between the regression and progression groups. Of note, the fibrotic areas appear greater than the lipidic areas in the progression group in this model, in agreement with the MRI.
Discussion

We have shown that serial MRI not only documents atherosclerotic plaque composition and burden in this model, but also allows the monitoring of changes over time and under different conditions of atherosclerotic lesions in the same animal.

MRI has been used to study atherosclerosis ex vivo and in vivo in both animal models and humans.11–13 We have previously shown that MRI can provide serial and noninvasive data about the arterial wall, allowing assessment of arterial remodeling in an experimental model.16 In this study, we have been able to document changes in aortic remodeling, in response to atherosclerosis progression and regression, that are consistent with this previously published work, although further study is clearly required to investigate the pathobiology of this process.

We have also demonstrated that in vivo MRI can reliably and noninvasively detect and quantify fibrous and lipid components of aortic atherosclerosis in a rabbit model, using the same imaging parameters as in this study.13 As in previous reports,11,15,17 the lipidic areas have a low signal on T2W and PDW images and are very distinctive from the fibrous areas that have a high signal on T2W and PDW images. In some sections in which the fibrous cap appears thin by histopathology, the MR image will tend to overestimate this component, as volume averaging with adjacent voxels will occur with the resolution used in this MRI sequence. However, despite these potential limitations, we have shown in this and previous studies15 that we are able to quantify these components with significant accuracy, although it is clear that further improvements in resolution would lead to greater accuracy in the measurement of atherosclerotic plaque composition.

The use of T2W imaging has emerged for the discrimination of fibrous and lipidic components of atherosclerotic lesions,9,10,13,18 although additional imaging sequences are required to assist with the differentiation of all the other components of human complex atherosclerotic lesions.9,12 The quantification of different components within the plaque is important because the individual makeup of the atherosclerotic plaque has been identified as a dominant prognostic factor. The experimental model used contains fibrotic and lipidic components only, and thus we are unable to comment on the ability of this MRI protocol to document serially changes in other atherosclerotic components. However, experimental and pathological data confirm the critical role the fibrotic and lipidic components play in atherosclerotic plaque vulnerability and thrombogenicity.4,7

A recent study has shown the potential of MRI to document the different levels of atherosclerotic burden in different rabbits subjected to different dietary regimens.13 However, serial data in the same animals at different time points were not obtained. Although the utility of serial MRI has been described in this model of atherosclerosis,11 we have shown...
that it is also possible to quantify changes in atherosclerotic and in lesion composition over time.

We have focused on the ability of a clinical magnet to evaluate serial noninvasive MRI to monitor changes in lipidic and fibrotic components of atherosclerotic plaques. Our results highlight the potential of MRI with a conventional coil on a whole-body 1.5-T clinical scanner to provide serial data about atherosclerotic lesions in patients at different time points and in response to therapeutic interventions. Future studies with serial MRI of human atherosclerosis are warranted to clarify its potential role.

MRI is able to document changes in atherosclerotic plaque size and composition in response to dietary modification in this experimental model of atherosclerosis. The potential exists for serial studies of human atheroma with MR in response to therapies such as lipid lowering.

Acknowledgments
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References
6. Fuster V, Badimon JJ. Regression or stabilization of atherosclerosis means regression or stabilization of what we don’t see in the arteriogram. Eur Heart J. 1995;16(suppl E):6–12.
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In the article “Progression and Regression of Atherosclerotic Lesions: Monitoring With Serial Noninvasive Magnetic Resonance Imaging” by Helft et al, which appeared in the February 26, 2002, issue of the journal (Circulation. 2002;105:993–998), an error appeared. The data in the first 2 columns of the Table (page 995) were reversed.

The corrected table appears below.

Serial MRI of Vessel Wall Parameters

<table>
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<tr>
<th></th>
<th>Lumen Area, mm^2</th>
<th>Mean Wall Thickness, mm</th>
<th>Vessel Wall Area, mm^2</th>
<th>Lipidic Area, mm^2</th>
<th>Fibrotic Area, mm^2</th>
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